Modeling the oscillation dynamics of activated airway smooth muscle strips

Jason H. T. Bates1 and Anne-Marie Lauzon2

1Vermont Lung Center, University of Vermont College of Medicine, Burlington, Vermont; and 2Meakins-Christie Laboratories, McGill University, Montreal, Quebec, Canada

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Bates, Jason H. T., and Anne-Marie Lauzon. Modeling the oscillation dynamics of activated airway smooth muscle strips. Am J Physiol Lung Cell Mol Physiol 289: L849–L855, 2005.—When strips of activated airway smooth muscle are stretched cyclically, they exhibit force-length loops that vary substantially in both position and shape with the amplitude and frequency of the stretch. This behavior has recently been ascribed to a dynamic interaction between the imposed stretch and the number of actin-myosin interactions in the muscle. However, it is well known that the passive rheological properties of smooth muscle have a major influence on its mechanical properties. We therefore hypothesized that these rheological properties play a significant role in the force-length dynamics of activated smooth muscle. To test the plausibility of this hypothesis, we developed a model of the smooth muscle strip consisting of a force generator in series with an elastic component. Realistic steady-state force-length loops are predicted by the model when the force generator obeys a hyperbolic force-velocity relationship, the series elastic component is highly nonlinear, and both elastic stiffness and force generation are adjusted so that peak loop force equals isometric force. We conclude that the dynamic behavior of airway smooth muscle can be ascribed in large part to an interaction between connective tissue rheology and the force-velocity behavior of contractile proteins.

computational model; force-velocity behavior; force-length behavior; connective tissue rheology

THE ABILITY OF AIRWAY SMOOTH MUSCLE to shorten is greatly influenced by the mechanical load against which it must contract. A significant component of this load is provided by the forces of airway-parenchymal interdependence (5), which change throughout the respiratory cycle. Mean airway caliber thus represents a dynamic balance between smooth muscle force and opposing load, and it has been suggested that this balance may become upset in asthma (27). Several investigators have recently explored this issue in vitro by subjecting strips of activated smooth muscle to oscillatory length changes (8, 20, 26). The resulting force-length behavior has been shown to depend in a rather complicated way on the amplitude and frequency of the oscillations (8, 9, 26), as well as on the duration over which the oscillations are applied (20, 26). Given that this behavior likely also pertains in situ, interpreting its genesis is important for furthering our understanding of the role played by airway smooth muscle in determining bronchial responsiveness.

Some investigators have developed mathematical models of the dynamics of activated airway smooth muscle as a means of dealing with the complexities involved. Anafi and Wilson (1) accurately captured the steady-state behavior of oscillated strips of activated airway smooth muscle in an empirical model but made no pretense of representing the mechanisms responsible for this behavior. A more mechanistic approach was taken by Fredberg and coworkers (9, 21), who attributed muscle strip dynamics entirely to strain-induced disruption of cross-bridge kinetics. However, neither of these models specifically takes into account the passive rheological properties of connective tissue. These properties have been shown to contribute significantly to the static stress-strain behavior of airway smooth muscle strips (24, 31). Accordingly, we hypothesized that tissue rheology should play a significant role in the dynamic mechanical behavior of activated strips of airway smooth muscle. To test the plausibility of this hypothesis, we modeled the smooth muscle strip as an active force generator connected to a series elastic component. In the present paper, we explore the extent to which this model is able to account for published experimental data.

COMPUTATIONAL METHODS AND RESULTS

Our goal is to develop a computational model of the activated airway smooth muscle strip that incorporates both muscle mechanics and tissue rheology, and this requires that we have experimental data against which the model can be validated. The data we have selected for this purpose come from the published literature. The first data set is the force-length behavior obtained by Fredberg et al. (8) in an activated strip of bovine trachealis oscillated sinusoidally at 0.33 Hz over a range of amplitudes. These data were collected after the strip had been oscillated at the amplitude in question for 2 h and therefore represent steady-state behavior (see Fig. 1 of Ref. 8) that was substantially different from the initial force-length behavior (20). The forces generated in the strip were out of phase with the length changes, giving rise to force-length loops rather than single-valued functions. Also, these loops were all obtained at the same mean strip length, so the maximum length increased with strain amplitude. The other data set we tested our model against consists of steady-state force-length loops collected by Shen et al. (26). In this case, the maximum muscle strip length was the same for each loop, so the mean length decreased with strain amplitude. One set of force-length loops (Fig. 2A of Ref. 26) was obtained when the strip length changed at the fixed rate of 0.02 Lo/s over various strain amplitudes, where Lo is the length at which maximum active force is generated. The other set of loops collected by Shen et al. (Fig. 2B of Ref. 26) was obtained at a fixed strain amplitude but with different rates of length change. In both sets of force-length loops collected by Shen et al. (26), the peak force in each loop was quite close to the maximal isometric force. The force-length loops collected by Fred-
berg et al. (8) and Shen et al. (26) are also reproduced in Anafl and Wilson (1).

We will now develop a simple mechanistically based model of the smooth muscle strip to account for the various features seen in the data identified above. Such a model obviously requires a force generator to represent the activity of the contractile proteins, actin and myosin, which are responsible for the ability of smooth muscle to shorten. In a muscle strip experiment, the activities of these contractile proteins are transmitted to force and length transducers through a substantial amount of connective tissue (19). This tissue contributes to what are known as the series and parallel elastic components of the muscle, which have been modeled together with the contractile component in vascular smooth muscle as either a Maxwell or a Voigt body (6). In the present study we consider relatively small variations in muscle strain, and so, following Seow and Stephens (25), we will assume that the changes in stress across the parallel elastic component play only a small role in the force-length loops of Fredberg et al. (8) and Shen et al. (26). We thus focus on the interactions between the contractile proteins of smooth muscle and the series elastic component and therefore model the oscillating activated smooth muscle strip as a force generator connected in series to a spring, as shown in Fig. 1A.

The next task is to assign properties to the two components in Fig. 1A. These properties could rapidly become highly complex, given what is known about the rheology of biological tissue (2, 10) and the biophysics of actin and myosin (4, 13, 29), but we will begin with the simplest properties that make physical sense. The simplest behavior we can assign to the series elastic component so that it maintains the integrity of the smooth muscle strip, while transmitting the generated forces to the measuring apparatus, is that of a Hookean spring. Thus, if $E$ is the elastic spring constant, $x(t)$ is the length of the spring, $t$ is time, $x_0$ is the relaxed spring length, and $F(t)$ is the force it sustains, then

$$F(t) = E[x(t) - x_0]$$

In the case of the force generator itself, we need to consider that activated smooth muscle generates a force that varies both with its length and with the rate of change of length. However, if the smooth muscle is stretched near its optimal length, then force varies with length only to second order. Furthermore, the peak in the force-length curve is rather broad (18), so we will assume for now that force is actually independent of smooth muscle length. The dependence of force on rate of change of length, however, cannot be neglected because force decreases markedly as contraction velocity increases (14, 28). This is depicted in Fig. 1B and captured in the following equation:

$$\frac{dy(t)}{dt} = \frac{b[F_0 - F(t)]}{a + F(t)} \text{ when } F(t) < F_0$$

$$= \frac{bF_0}{a} - \frac{bF(t)}{a + F_0} \text{ when } F(t) \geq F_0$$

where $y(t)$ is the length of the force-generating unit, $a$ is a constant with units of force, $b$ is a constant with units of velocity, and $F_0$ is isometric muscle force. The first line of Eq. 2 is the hyperbolic Hill equation that has been described for airway smooth muscle by various investigators (3, 14, 28). The second line of Eq. 2 is the equation of a straight line that accounts for eccentric contraction of the smooth muscle and has the same slope as the hyperbolic relationship at a velocity of zero in accord with the experimental findings of Hanks and Stephens (14).

Equations 1 and 2 constitute our basic model of the smooth muscle strip, which we implement numerically as follows. Initially, the smooth muscle strip is relaxed and uncontracted, so its length is $y_0 + x_0$ and $F$ is zero. At $t = 0$ we activate the contractile component and impose a sinusoidal oscillation of frequency $f$ Hz on the right-hand end of the series elastic component (Fig. 1A), while keeping the left-hand end of the contractile component fixed, such that

$$x(t) + y(t) = x_0 + y_0 + A \sin(2\pi ft)$$

Initially, there is no load on the smooth muscle so it contracts [i.e., $y(t)$ decreases] with the maximum possible velocity of $bF_0/a$ (Eq. 2). However, as the contractile component shortens and the series elastic component is pulled to the right, the spring becomes extended and generates a force that is transmitted to the contractile component and changes its velocity of shortening. This determines the rate of change of $y$ according to Eq. 2, from which the value of $y$ one time-step later is determined by numerical integration. We solved this system of equations by first-order Euler integration with a time-step of 5 ms. Each simulation was run for 50 s to allow the model to reach steady-state behavior.

We first endeavored to have the model match the force-length loops of Fredberg et al. (Fig. 1 of Ref. 8) by oscillating

![Image 1A](http://ajplung.physiology.org/) (by 10.220.33.5 on June 27, 2017)
the model sinusoidally at the same frequency of 0.33 Hz over
the same strain excursions of 0.25, 0.5, 1, 2, 4, and 8%. We
used the following values for the model parameters: \(a = 0.25,\)
\(b = 0.5, F_0 = 1, E = 0.5, x_0 = 50, y_0 = 0\) (all quantities in
arbitrary units). Apart from requiring that \(a/F_0 = 0.25\) to
conform to experimental observation (3), these values were
identified by trial and error and produce force-length loops that
become progressively wider with amplitude (Fig. 2A) but
which remain elliptical as amplitude increases. By contrast, the
experimental loops become progressively more “banana
shaped” with increasing amplitude (see Fig. 1 of Ref. 8),
suggesting the influence of some nonlinear mechanical
property. Indeed, Seow and Stephens (25) found stress in the series
elastic component of tracheal smooth muscle to depend expo-
entially on strain, suggesting that the spring in Fig. 1A should
be non-Hookean. We therefore made the stiffness of the spring
highly nonlinear. In the interest of keeping the number of free
parameters in the model to a minimum, we chose the form of
the nonlinearity to be

\[ F(t) = E[(x(t) - x_0)^4] \]

This function replaces Eq. 1 and has only a single parameter,
\(E\), which we set to 0.005 (again in arbitrary units). This gives
rise to force-length loops that become progressively more
nonlinear in shape as strain increases (Fig. 2B).

However, this still does not make the model behave like real
muscle tissue because the maximum loop force in Fig. 2B
increases markedly with strain amplitude while the experi-
mental loops exhibit a maximum force that varies little with
amplitude (Fig. 1 of Ref. 8). Shen et al. (26) also found a
constant peak loop force when they varied strain amplitude but
kept maximum strip length fixed. Together, these data imply
that, given enough time, a strip of activated smooth muscle is
somehow able to adjust its peak oscillatory force to be close to
isometric tension, regardless of the amplitude or frequency of
oscillation. Indeed, substantial reductions in peak tension with
continued oscillation are clearly demonstrated in the data of
Latourelle et al. (20), who ascribed this behavior to the pro-
gressive perturbation of the binding of myosin to actin. How-
ever, it could equally reflect passive stress adaptation within
the connective tissue of the strip, as all biological soft tissue is
viscoelastic and exhibits marked stress adaptation over a huge
range of time scales (2). This adaptation presumably occurs
because the constituents of the tissue rearrange themselves in
response to a change in strain to minimize the resulting stress.

When strain is large, such adaptation might allow some of the
constituents to move from a parallel juxtaposition to a more
serial one, or even to disengage from other elements altogether,
leading to a progressive lengthening of the series elastic com-
ponent with a concomitant decrease in its stiffness. Such
lengthening would also lead to increased shortening of the
contractile apparatus, possibly to the point where the contrac-
tile fibers could encounter physical impediments to further
shortening, as has been suggested in the context of the plas-
ticity theory of smooth muscle (11, 26).

The above reasoning suggests that both the maximum
steady-state force of the contractile unit and the stiffness of the
series elastic component should decrease with repeated oscil-
lations until the maximum force in the tissue is similar to \(F_0\).
We achieved this in the model as follows. In each oscillatory
cycle we determined the force difference, \(\Delta F\), between the
maximum simulated force and isometric tension. The param-
eters \(F_0, a,\) and \(E\) were then scaled by the factor \(F_0/(F_0 + \Delta F)\)
before being used to simulate the following cycle. This caused
the maximum force in each loop to asymptote toward isometric
tension and produced the force-length loops shown in Fig. 2C,
which resemble the experimental loops obtained by Fredberg et
al. (Fig. 1 in Ref. 8) rather closely.

So far, we have succeeded in developing a model of the
activated smooth muscle strip that can accurately reproduce

Fig. 2. A: simulated force-length loops obtained at 0.25, 0.5, 1, 2, 4, and 8%
sinusoidal strain at a frequency of 0.33 Hz with a Hookean spring in the tissue
strip model (Fig. 1A). B: simulated loops obtained when the Hookean spring
was replaced with a nonlinear spring (Eq. 4). C: simulated loops when the
model was further modified so that peak loop force equaled isometric force. All
loops proceed in a clockwise fashion.
steady-state force-length loops under one particular set of circumstances, namely sinusoidal oscillation at various amplitudes about a fixed mean length. An important test of the model is now to see how well it can account for a different data set. The force-length loops of Shen et al. (26) provide an appropriate opportunity. These loops differ from those of Fredberg et al. (8) in two important respects: the rate of change of muscle length was constant rather than sinusoidal, and in each set of force-length loops the peak length was fixed. We first obtained steady-state loops from the model using the same velocities, amplitudes, and frequency as in Ref. 26 and with the same parameter values as used to generate the simulated loops in Fig. 2C. The results are shown in Fig. 3A and exhibit many of the key features of the experimental data of Shen et al. (Fig. 2A of Ref. 26). In particular, the descending limbs of the loops all follow a similar trajectory while the ascending limbs move leftward with increasing amplitude. However, the simulated loops are rather too wide compared with the experimental data in Ref. 26. We obtained more realistic looking loops by reducing the initial value of $b$ from 0.5 to 0.25 (Fig. 3B). The parameter $b$ scales the contraction velocity of the muscle, so reducing its value can be viewed as simulating a reduction in the excitation level of the muscle, equivalent to reducing the number of contracting proteins linked in series. We then applied the same model to the description of the data of Shen et al. (Fig. 2B of Ref. 26) by oscillating it at the same amplitude and frequencies. Although somewhat wider than the experimental loops, the simulated loops exhibit the same leftward movement and elevation in minimum force as frequency decreases (Fig. 3C).

**DISCUSSION**

The key events in airway smooth muscle contraction are still a matter of some debate due in part to the rather complicated way in which activated strips of smooth muscle respond to changes in length and load. Anafi and Wilson (1) adopted a purely empirical approach to the modeling of smooth muscle dynamics and were able to accurately reproduce experimental force-length behavior data from smooth muscle strips. Their goal was to devise a purely predictive tool, which they demonstrated convincingly by simulating the response of a constricted airway to deep inspiration. Anafi and Wilson (1) made no pretense of incorporating underlying mechanisms into their model. Interestingly, though, they did point out that some of the terms in their model equations represent nonlinear springs and relaxation processes, both of which are readily invoked in the description of the complex and nonlinear rheological behavior of biological soft tissue (2).

A mechanistic approach to the description of airway smooth muscle dynamics was taken by Fredberg’s group (21), who ascribed the looping of the steady-state force-length relationship of bovine trachealis entirely to an influence of strain on cross-bridge kinetics. That model is somewhat less accurate in its predictions of experimental data than the model of Anafi and Wilson (1) but potentially provides much greater insight into the factors regulating smooth muscle contractility. However, the Fredberg model (21) assumes that the strain profile applied to a strip of trachealis muscle impinges directly on the cross-bridges within the smooth muscle cells. This model thus considers the smooth muscle strip to effectively behave like a single pair of actin and myosin filaments oriented with the direction of strain. Changes in the ensemble attachment/detachment behavior of the cross-bridges as strain varies give rise to force-length looping in this model. However, although such effects on cross-bridge kinetics have indeed been described in skeletal muscle (29) and may also occur in smooth muscle.
scribing all the dynamics of the smooth muscle strip to this mechanism neglects any influence due to the passive mechanical properties of the noncontractile tissue within the smooth muscle strip. In particular, the contractile proteins transmit their activity through the series elastic component to the experimental apparatus that measures stress and strain. It therefore seems unreasonable not to include the influence of passive mechanical properties in a model of activated smooth muscle dynamics.

This raises the question of how the passive mechanical properties of the muscle should be modeled. The elastic properties of smooth muscle tissue are conventionally attributed to both series and parallel components (6). Our model (Fig. 1A) does not include the parallel elastic component, yet the importance of this component in airway smooth muscle has been demonstrated by stress-strain measurements on relaxed muscle and reveals a markedly nonlinear behavior that varies between species (24). However, Seow and Stephens (25) point out that when the strain variation of the muscle is relatively small, the parallel elastic component likely has little effect on the interaction between the contractile proteins and the series elastic component.

A related question is whether we are justified in considering the series elastic component to be structurally distinct from the contractile proteins themselves. Whereas in skeletal muscle the series elastic component is closely associated with the number of attached cross-bridges, in smooth muscle the situation is much less clear (25). Indeed, there is little agreement as to the anatomical location of the series elastic component. Nevertheless, while some investigators have found this component to lie largely within the smooth muscle cell (23), others have concluded that it is not associated with the contractile apparatus (6). These various considerations led us to conclude that the dynamic force-length behavior of activated smooth muscle strips should be modeled in the broadest terms as a contractile component in series with an elastic component.

As a first step, we represented the series elastic component as a Hookean spring. We modeled the dependence of force on velocity for the contractile component as shown in Fig. 1B because this behavior has been demonstrated experimentally by numerous investigators in quick-release experiments (3, 14, 22, 28, 31). Whether this is really appropriate for oscillating muscle strips was concluded in bovine trachealis stimulated with 10^{-5} M acetylcholine, whereas those of Fredberg et al. (8) were obtained in canine trachealis stimulated with 10^{-4} M acetylcholine, so there is no reason to suspect that these two data sets

However, the persistently elliptical shape of the force-length loops obtained with the Hookean spring (Fig. 2A) is at odds with the experimental loops, which become progressively more bent as amplitude increases (Fig. 1 of Ref. 8). We reasoned that this likely reflects the kind of nonlinearity in stiffness seen in all types of biological soft tissue. Indeed, Seow and Stephens (25) observed nonlinear stress-strain behavior in the series elastic component of trachealis, so we next incorporated a highly nonlinear spring in the model. This gave the appropriate degree of curvature to the force-length loops (Fig. 2B). However, the model still failed to account for the fact that the experimental loops (Fig. 1 of Ref. 8) exhibit peak forces that vary little with oscillation amplitude, despite substantial differences in the maximum length of the muscle strip between the different loops.

This constancy of peak force found by Fredberg et al. (8) is a curious phenomenon, as one would normally expect that an increase in peak length should result in an increase in peak force. What is even more surprising is that peak force also remains constant when oscillation amplitude is varied without a change in peak length, as shown by Shen et al. (26). However, the data reported by both Fredberg et al. (8) and Shen et al. (26) are all steady-state force-length loops obtained after the muscle strips were oscillated for several minutes. Initially, the force-length loops reached significantly higher forces than in these steady-state loops (20). Thus some relaxation process must have occurred within the muscle tissue that caused its peak force-generating capacity to asymptote toward a fixed level close to maximum isometric force, regardless of how the tissue was stretched. Fredberg et al. (9) implicated plastic remodeling of the cytoskeleton in this phenomenon and have even suggested that such behavior makes it appropriate to classify smooth muscle as a glassy material (7). Anaft and Wilson (1) also implicated relaxation processes in their modeling study, and indeed stress adaptation takes place in all manner of biological soft tissue over a huge range of time scales (2). We do not include stress adaptation explicitly in our model of the smooth muscle strip, but we invoke its effects to account for the constancy of peak force by progressively adjusting the parameters determining F_0, velocity (a), and tissue stiffness (E) until peak loop force matched maximum isometric force. This mimics what would be expected if the spring in our model (Fig. 1A) were viscoelastic, giving it the ability to exhibit creep, instead of being purely elastic. Indeed, we could presumably make this element assume the glassy behavior that has been attributed to smooth muscle (7) by assigning the spring an appropriate complex rheology. In any case, the ad hoc relaxation process we utilized in our model produces force-length loops (Fig. 2C) that look very similar in many respects to those obtained experimentally by Fredberg et al. (Fig. 1 of Ref. 8).

The next step in validating the model was to apply it to a different data set, namely that of Shen et al. (26). When we employed the model to simulate the data shown in Fig. 2A of Shen et al. (26) using the same parameter values that generated Fig. 2A of the present study, the resulting force-length loops were too wide (Fig. 3A). However, the data of Shen et al. (26) were obtained in canine trachealis stimulated with 10^{-5} M acetylcholine, whereas those of Fredberg et al. (8) were obtained in bovine trachealis stimulated with 10^{-4} M acetylcholine, so there is no reason to suspect that these two data sets
should be mimicked by exactly the same computational model. In particular, the lower dose of agonist used for the data of Shen et al. (26) suggests that they might be better reproduced by the model if the activation of the contractile component was reduced somewhat. Accordingly, we reduced the value of the parameter $b$ by a factor of 2 (chosen arbitrarily) to mimic a reduced velocity of shortening. The resulting simulated loops shown in Fig. 3B are still not perfect reproductions of the experimental data of Shen et al. (Fig. 2A of Ref. 26), but they are significantly improved over those in Fig. 3A, and in particular the key features of the experimental data have been reproduced. These features include the leftward movement of the lengthening limbs, the similar trajectory of the shortening limbs, and the increasing curvature of the loops with increasing amplitude. As a final test of the model, we applied it to the data shown in Fig. 2B of Ref. 26, using the parameter values employed in generating Fig. 3B. The results shown in Fig. 3C are also not perfect renditions of the corresponding experimental loops in Fig. 2B of Ref. 26, but they again capture the essential features of the data, including a leftward shift of the lengthening limb and an increase in minimum force as frequency decreases.

The remaining shape differences between the experimental data and our simulations could be due, at least in part, to the behavior of viscoelastic elements in the smooth muscle tissue. We did not include such elements in our model to keep it simple, but combinations of viscous and elastic elements could easily contribute to force-length looping in smooth muscle (16, 17). Another omission from our model was an active force-generating capacity that depends on muscle length. Active force is known to reach a maximum at a length conventionally identified as $L_0$ and to decrease progressively as length moves either side of $L_0$ (24). However, the maximum strain we simulated in this study was only 10%, which corresponds roughly to a 10% change in isometric force-generating capacity in smooth muscle from a variety of species (24). Thus, although inclusion of length variation in active force might have changed the shape of our simulated loops somewhat, the differences would be small and would not affect our conclusions in any way. Finally, it has recently been shown that smooth muscle can change its optimal length for force generation depending on the length at which it is initially stimulated (11, 12, 15). This plasticity mechanism may have significant implications for the function of airway smooth muscle in vivo, but for the purposes of the present study it probably plays little role because the experimental force-length data that we have attempted to simulate were obtained following careful standardization of length and stimulation history (8, 26).

Finally, although we have managed to show that the interaction between an active force generator and a series elastic element can, in principle, account for the main features of published force-length loops in activated muscle strips, this in no way proves that our model is correct. It therefore behooves us to at least contemplate how the model might be further tested. One possibility would be to alter the series elastic element in a strip of smooth muscle and see if the changes this produces in activated force-length looping can be accounted for by an appropriate change in the spring constant in our model. For example, treatment with an enzyme that degrades connective tissue would be expected to decrease the stiffness of that part of the series elastic element external to the cell. Our model predicts that this would cause a decrease in the width of the force-length loops. Alternatively, one could test the predictions of the model against force-length loops measured in smooth muscle strips that have altered contraction velocity, such as from mice missing the fast isoform of myosin (30).

In conclusion, we have developed a computational model of the activated smooth muscle strip consisting of a force generator, representing the contractile proteins, in series with a passive elastic component, representing the connective tissue. The shortening velocity of the force generator is finite and decreases monotonically as load increases with elongation of the series elastic component. The model simulates steady-state force-length loops that show key similarities in shape to those observed experimentally. Our findings demonstrate thus that the principle features of the force-length behavior of activated smooth muscle strips can be understood in terms of the force-velocity behavior of contractile proteins interacting with the mechanical properties of the series elastic component.

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